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IFN-α Enhances IL-22 Receptor Expression in Keratinocytes: A Possible Role in the Development of Psoriasis

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TO THE EDITOR

Recently, a pathological role for IFN-α in the development of psoriasis has been suggested. Clinically, exacerbation of psoriasis has often been observed after starting IFN-α therapy for other diseases. IFN-α induced by topical application of a Toll-like receptor-7 agonist can also trigger the development of psoriasis (Gilliet *et al.*, 2004). Histopathologically, plasmacytoid dendritic cells (pDCs), the principal IFN-α–producing cells, infiltrate into the psoriatic lesions in the early phase (Wollenberg *et al.*, 2002; Gilliet *et al.*, 2004). In a xenograft model, in which human psoriatic skin was transplanted onto immunodeficient mice, Nestle *et al.* (2005) clearly demonstrated that blocking IFN-α signaling prevents the T cell–dependent development of psoriasis and that inhibiting the production of IFN-α by pDCs completely suppresses the development of a psoriatic phenotype. IFN-α may mediate the activation of T cells directly or through the induction of myeloid dendritic cell activation and/or maturation. The involvement of keratinocytes, however, remains unclear.

We recently reported that IL-22 receptor (IL-22R) expression is enhanced in the epidermis of psoriasis compared with normal skin (Tohyama *et al.*, 2009). IL-22, a member of the IL-10 family, is one of the most important cytokines in the pathogenesis of psoriasis, contributing to proliferation/differentiation of keratinocytes and to the production of antimicrobial peptides, cytokines, and chemokines (Wolk *et al.*, 2006; Sa *et al.*, 2007). Receptors for IL-22 include IL-22R and IL-10 receptor 2 (IL-10R2). IL-10R2 is widely expressed on various cells and tissues. On the other hand, IL-22R expression is limited mainly to epithelial cells, including epidermal keratinocytes, where IL-22R expression levels are altered by cytokine stimulation. Increased IL-22R expression strengthens the responsiveness of keratinocytes to IL-22 stimulation (Tohyama *et al.*, 2009). These findings suggest that an increase in IL-22R expression on epidermal keratinocytes is an important feature in psoriasis.

We hypothesized that IFN-α enhances IL-22R expression on epidermal keratinocytes. To examine this, we used a living skin equivalent (LSE) model (Yang *et al.*, 2005), as IL-22R expression of cultured keratinocyte monolayers is suppressed relative to that of stratified epidermal keratinocytes, including those in the LSE (Sa *et al.*, 2007). To prepare the LSE, normal human epidermal keratinocytes and fibroblasts were isolated from nonlesional human skin obtained from plastic surgery and then cultured. A collagen gel containing the fibroblasts was prepared, and the keratinocytes were seeded onto the concave surface of the contracted gel. When the keratinocytes reached confluence, the LSE was raised to the air–liquid interface, and cornification medium was added. Experiments were conducted from 8 to 12 days after air-lifting. Total RNA or protein was extracted from the LSE epidermis for real-time reverse transcription-PCR analysis or western blotting, respectively. For immunofluorescence staining, frozen LSE sections (5 μm) were fixed in cold acetone or methanol and reacted with primary antibodies. Sections were then incubated with donkey anti-rabbit or anti-goat antibodies labeled with Alexa Fluor 488 (Molecular Probes, Eugene, OR). Alternatively, LSE epidermis was treated with trypsin, and flow cytometry analysis (FCM) was performed as described previously (Tohyama *et al.*, 2009). For each examination, results were confirmed in three independent experiments.

LSE was treated with IFN-α for 4, 8, or 24 hours. IFN-α markedly enhanced IL-22R mRNA expression in the epidermis after 4 hours of stimulation (Figure 1a), and this increase was dose dependent (Figure 1b). In contrast, IL-10R2 mRNA expression level was not changed (Figure 1c). The expression of IL-20R1 and IL-20R2, receptors for IL-10 family cytokines other than IL-22, was also unaffected by IFN-α.

LSE treated with or without IFN-α for 24 hours was snap-frozen, and immunofluorescence staining was performed using anti-IL-22R antibody (R&D systems, Minneapolis, MN). IL-22R expression was increased relative to controls in the LSE epidermis treated with IFN-α (Figure 1d). Increased IL-22R expression on keratinocyte cell surfaces was also observed by FCM analysis (Figure 1e).

To examine whether the response to IL-22 is increased by IFN-α treatment, phosphorylation of signal transducer and activator of transcription 3 (STAT3) was examined by western blotting. Anti-phospho-STAT3 antibody was purchased from Cell Signaling Technology (Danvers, MA). As shown in Figure 2a, STAT3 was phosphorylated at 30 and 60 minutes after IL-22 stimulation in the epidermis of LSE. When LSE was pretreated with IFN-α for 24 hours, phosphorylation of STAT3 was markedly augmented.

Abbreviations: FCM, flow cytometry; IL-10R, IL-10 receptor; IL-22R, IL-22 receptor; LSE, living skin equivalent; pDC, plasmacytoid dendritic cell; STAT3, signal transducer and activator of transcription 3

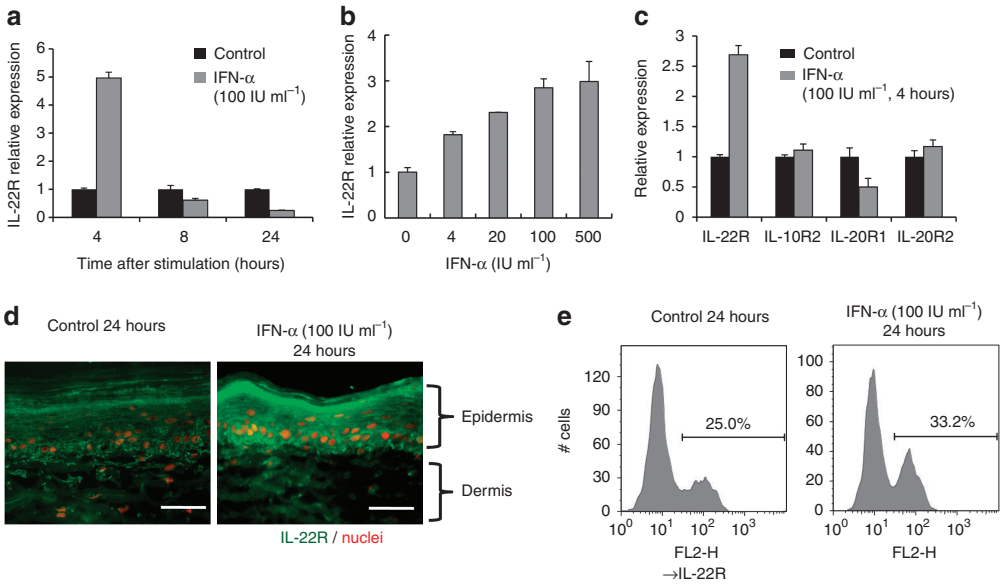

Figure 1. Enhancement of IL-22 receptor (IL-22R) expression in keratinocytes by IFN-α. Living skin equivalent was treated with IFN-α for the indicated time and at the indicated concentrations (**a**–**e**). mRNA expression of IL-22R (**a**, **b**) or other receptors (**c**) was analyzed and normalized to glyceraldehyde-3-phosphate dehydrogenase control. The results are expressed as the mean ± standard deviation. IL-22R protein expression in keratinocytes was analyzed by immunofluorescence staining (**d**) or by flow cytometry analysis (**e**). Bar = 100 μm.

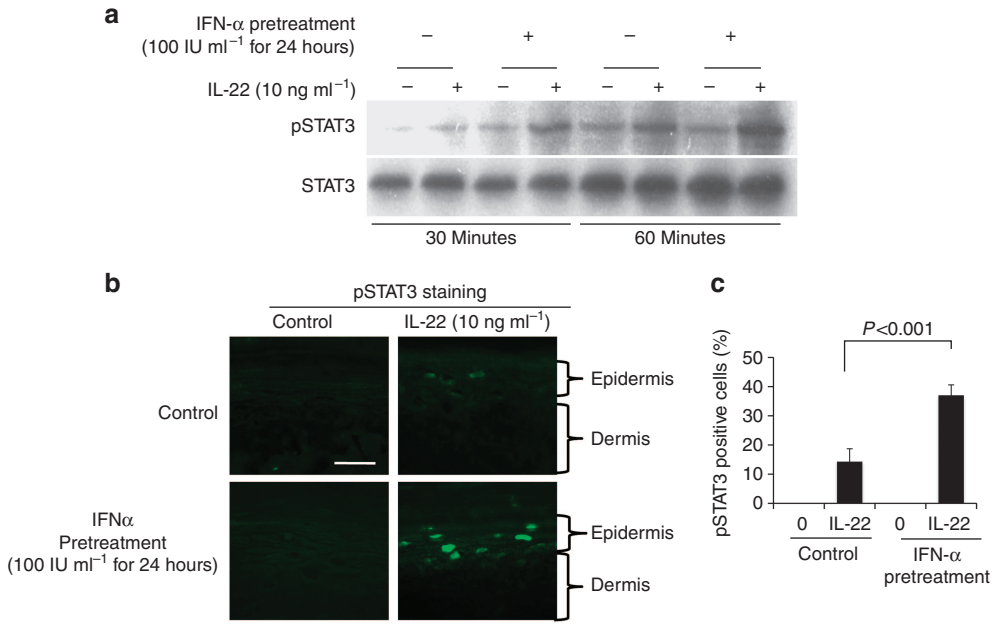

Figure 2. Enhancement of IL-22 receptor (IL-22R)-induced signal transducer and activator of transcription 3 (STAT3) phosphorylation by IFN-α. Living skin equivalent (LSE) was pretreated with IFN-α for 24 hours and then stimulated with IL-22 for 30 and 60 minutes (**a**) or for 60 minutes (**b**). pSTAT3 was analyzed by western blotting (**a**) or immunofluorescence staining (**b**). Nuclear staining of pSTAT3 was apparent in the keratinocytes of LSE pretreated with IFN-α (**b**). Bar = 100 μm. Percentage of nuclear pSTAT3-positive cells was measured, and the results are expressed as the mean ± standard deviation (**c**). Statistical analysis of the data was evaluated using Student's *t*-test.

Immunostaining revealed that phospho-STAT3 appeared in the nuclei of IFN-α-pretreated keratinocytes after IL-22 stimulation, whereas staining was weak and the percentage of phospho-STAT3-positive cells was low in untreated keratinocytes, even after IL-22 addition (Figure 2b and c).

We clearly demonstrated here that IFN-α strengthens the responsiveness of epidermal keratinocytes to IL-22 via increased IL-22R expression. These findings explain the exacerbation of psoriasis due to IFN-α therapy and support the theory that IFN-α triggers the development of psoriatic lesions. However, this mechanism is not specific to psoriasis. IL-22 is required for

host defenses against microbial infection, inflammation, and injury (Ouyang, 2010). Increasing IL-22R expression in keratinocytes by IFN-α may be one of the defense systems of injured skin. Damage causes infiltration of pDCs, which are activated by self-nucleic acid derived from damaged keratinocytes or other cells via Toll-like receptors 7 and 9, producing IFN-α/β (Gregorio *et al.*, 2010). Although the detailed mechanisms remain unclear, IFN-α/β produced by pDCs influences IL-17- and IL-22-producing T cells by activation of myeloid dendritic cells (Gregorio *et al.*, 2010). IL-22 signals via IL-22R inhibit keratinocyte terminal differentiation, causing thickening of the epidermis (Sa *et al.*, 2007). IL-22 also induces production of heparin-binding epidermal growth factor-like growth factor from keratinocytes and may mediate the proliferation of the epidermis (Sa *et al.*, 2007; Tohyama *et al.*, 2009). Moreover, IL-22 induces expression of several genes in keratinocytes, such as human β-defensin 2 and S100 family genes, that are antimicrobial proteins (Wolk *et al.*, 2006; Ouyang, 2010). Although these cellular responses will usually become downregulated after the repair of injury, the responses may trigger the development of lesions in psoriasis. Further studies will be necessary to clarify this mechanism.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at http://www.nature.com/jid

REFERENCES

Gilliet M, Conrad C, Geiges M *et al.* (2004) Psoriasis triggered by Toll-like receptor 7 agonist imiquimod in the presence of dermal plasmacytoid dendritic cell precursors. *Arch Dermatol* 140:1490–5

Gregorio J, Meller S, Conrad C *et al.* (2010) Plasmacytoid dendritic cells sense skin injury and promote wound healing through type I interferons. *J Exp Med* 207:2921–30

Nestle FO, Conrad C, Tun-Kyi A *et al.* (2005) Plasmacytoid predendritic cells initiate psoriasis through interferon-alpha production. *J Exp Med* 202:135–43

Ouyang W (2010) Distinct roles of IL-22 in human psoriasis and inflammatory bowel disease. *Cytokine Growth Factor Rev* 21:435–41

Sa SM, Valdez PA, Wu J *et al.* (2007) The effects of IL-20 subfamily cytokines on reconstituted human epidermis suggest potential roles in cutaneous innate defense and pathogenic adaptive immunity in psoriasis. *J Immunol* 178:2229–40

Tohyama M, Hanakawa Y, Shirakata Y *et al.* (2009) IL-17 and IL-22 mediate IL-20 subfamily cytokine production in cultured keratinocytes via increased IL-22 receptor expression. *Eur J Immunol* 39:2779–88

Wolk K, Witte E, Wallace E *et al.* (2006) IL-22 regulates the expression of genes responsible for antimicrobial defense, cellular differentiation, and mobility in keratinocytes: a potential role in psoriasis. *Eur J Immunol* 36:1309–23

Wollenberg A, Wagner M, Gunther S *et al.* (2002) Plasmacytoid dendritic cells: a new cutaneous dendritic cell subset with distinct role in inflammatory skin diseases. *J Invest Dermatol* 119:1096–102

Yang L, Shirakata Y, Tamai K *et al.* (2005) Microbubble-enhanced ultrasound for gene transfer into living skin equivalents. *J Dermatol Sci* 40:105–14

The Mutation Profiles of Common Oncogenes Involved in Melanoma in Southern China

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TO THE EDITOR

Significant advances in the understanding of the biology and molecular mechanisms of cancer have allowed the development of new targeted agents for the treatment of melanoma. Previous studies showed that *BRAF* and *NRAS* mutations were observed in approximately 40–60% and 15–20% of cutaneous melanomas (Chapman *et al.*, 2011; Lee *et al.*, 2011), respectively. Most of the data regarding genetic mutations associated with melanomas have been obtained from Caucasian cohorts, and comprehensive screenings of the Chinese population are limited. In this study, we examined the mutation profiles using the MassARRAY System (Sequenom, San Diego, CA) to determine the prevalence of oncogene mutations in melanoma patients from southern China.

The MassARRAY platform provides a superior technology for the screening of 238 hot-spot cancer mutations in 19 common oncogenes: *ABL1*, *AKT1*, *AKT2*, *BRAF*, *CDK4*, *EGFR*, *ERBB2*, *FGFR1*, *FGFR3*, *FLT3*, *JAK2*, *KIT*, *MET*, *HRAS*, *KRAS*, *NRAS*, *PDGFRA*, *PIK3CA*, and *RET* (Supplementary Table S1 online). A total of 114 formalin-fixed and paraffin-embedded melanoma lesions were examined: 28 paired primary acral melanomas with corresponding lymph node metastasis; 28 mucosal melanomas; and 30 non-chronic sun-induced damage melanomas (Supplementary Table S2–4 online). The study protocol was approved by the research ethics committee of Sun Yat-Sen University Cancer Center, China.

At least one mutation was detected in 33 of the 86 (38.4%) melanomas, with mutations observed in *BRAF* (16.3%), *NRAS* (10.5%), *KIT* (5.8%), *EGFR*